

Chapter 7

The soft coral *Sinularia flexibilis*: potential for drug development

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ABSTRACT

Evidence available to date suggests that the soft coral *Sinularia flexibilis* (Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) offers a rich reserve of novel organic molecules, which can be useful as new drugs to combat diseases or as biochemical, physiological and pharmacological tools in biomedical research. In this article, over 210 studies until 2007 on the secondary metabolites isolated from known and unknown species of the genus *Sinularia* are reviewed. A total of 42 studies about compounds from *S. flexibilis* are listed. Several compounds with special or selective activities are described in more detail. It is important to investigate whether the compounds from *S. flexibilis* could be developed into future medical and industrial products. Cultivation of *S. flexibilis* under controlled conditions could be the solution to supply the biomass for pharmacological exploitation of some highly potent bioactive compounds.

INTRODUCTION

Among Cnidaria (formerly Coelenterata), 21 % of the species contain potential marine biomedical compounds (Jha and Zi-rong, 2004). Almost 50 % of soft corals (Octocorals) as members of Cnidaria (phylum Cnidaria, class Alcyonaria, subclass Octocorallia have been reported to produce toxins; about 60% of their extracts are bioactive molecules with medicinal potential (Coll *et al.*, 1982a; Coll, 1992; Higa *et al.*, 2001; Sheu *et al.*, 2002). It has been stated that those compounds are promising to be used against diseases without the shortcomings of steroids and other anti-inflammatory drugs that are presently used as medicines (Scripps, 2007).

As they lack physical defenses, soft-bodied sessile invertebrates such as soft corals often use a refined chemical weapon; they have been the first target in screening programs for bioactive compounds because of their potential to provide molecules of use in pharmacology and as antifouling agents (e.g. Coll, 1992; Temraz *et al.*, 2006). Octocorals (class Anthozoa,

subclass Octocorallia, order Alcyonacea, family Alcyoniidae) were one of the first marine groups that were systematically screened for secondary metabolites (Tursch, 1976). These compounds, especially cembranoid diterpenes (Hirono *et al.*, 2003), have a function in chemical defense, in competition for space (allelopathy), against fouling and they inhibit reproduction of other organisms such as fishes and some genera of hard corals (*Acropora*, *Porites*, *Pavona*) (e.g. Bowden *et al.*, 1985; Coll *et al.*, 1982a; Coll *et al.*, 1982b; Coll and Sammarco, 1983; Coll, 1992; Gerhart and Coll, 1993; Kamel *et al.*, 2007a; Kelman *et al.*, 2006; La Barre *et al.*, 1986; Ojika *et al.*, 2003; Sammarco *et al.*, 1983; Sheu *et al.*, 2002; Webb and Coll, 1983; Webb, 1986). For instance, stunting of growth in *Pavona cactus* occurred up to 30 cm away from the base of *S. flexibilis*. In addition, corals produce mycosporine-like amino acids (MAAs) and other mycosporines referred to as true 'multipurpose' secondary metabolites. The most important function of MAAs and

other mycosporines in nature is that they have a role as sunscreen against UV light (e.g. Oren and Gunde-Cimerman, 2007) in combination with other functionalities such as prevention of oxidation reactions (Shick and Dunlap, 2002). From all marine-derived potential new drugs in preclinical stage in 1998, 2001 and 2002, 11–17 % originate from soft corals (Mayer, 1999; Mayer and Lebmann, 2000; Mayer and Gustafson, 2003; 2004). This shows that soft corals are an important source of active biological molecules and model compounds for drugs (Carte, 1996; Coll *et al.*, 1985; Sato *et al.*, 1985). Other relevant organisms include sponges, mussels, snails, tunicates, bryozoans and fungi.

The soft coral genus *Sinularia* is one of the most widely distributed soft corals. About 60 % of sinularian corals contain toxins (e.g. Coll, 1992), including sesquiterpenes, diterpenes, norditerpenes, polyhydroxylated steroids, polyamine compounds with antimicrobial, anti-inflammatory and cytotoxic activities (Ahmed *et al.*, 2004a; Ahmed *et al.*, 2004b; Ahmed *et al.*, 2007; Bhosale *et al.*, 2002; Blunt *et al.*, 2006; Bowden *et al.*, 1978; Bowden *et al.*, 1981; Goto *et al.*, 1992; Hirono *et al.*, 2003; Jia *et al.*, 2006; Jin *et al.*, 2005; Kumar and Lakshmi, 2006; Li *et al.*, 2005; Liyanage *et al.*, 1992; Ojika *et al.*, 2003; Radhika *et al.*, 2002; Radhika *et al.*,

2004; Radhika *et al.*, 2005; Redy *et al.*, 2002; Sato *et al.*, 1985; Sheu *et al.*, 2002; Shindo *et al.*, 1992; Su *et al.*, 2000; Su *et al.*, 2005; Su *et al.*, 2006a; Su *et al.*, 2006b; Takaki *et al.*, 2003; Zhang *et al.*, 2005; Zhang *et al.*, 2007). Our extensive literature review over a period of more than 30 years has recorded 50 known species (out of a total of 90 species: Yu *et al.*, 2006) and 23 unknown species of *Sinularia* spp. that have been chemically examined. To the extent of our review, over 210 papers have been published about the chemical constituents of *Sinularia* (both from known and unknown species), the majority of which report novel cytotoxic terpenoids.

The soft coral *Sinularia flexibilis* (Figure 1) is cosmopolitan in its distribution and occurs in different seas (Anjaneyulu *et al.*, 1998; Coll, 1992). Chemical examination of several collections of this species led to the earliest isolation of a range of cembranoid diterpenes (e.g. Bowden *et al.*, 1992; Campos *et al.*, 1995; Su *et al.*, 2005; Hamade *et al.*, 1992) with potential anticancer activity (Tursch *et al.*, 1975). The current paper reviews the secondary metabolites of *S. flexibilis*, their biological and pharmacological significance, and various means of biomass supply for drug development.



Figure 1: A colony of *Sinularia flexibilis*.

SECONDARY METABOLITES OF *S. FLEXIBILIS* AND THEIR BIOACTIVE PROPERTIES

Table 1 lists all studies done on the bioactive

compounds of *S. flexibilis* with their biological activities from 1975 up till today.

In a previous review on the genus *Sinularia*, eight terpenoids of *S. flexibilis* in 15 studies from 1978 to 2002 were surveyed (Kamel and

Table 1: Studies on the secondary metabolites of the soft coral Sinularia flexibilis

Metabolite	Activity	Ref.
7,8-epoxy-11-epi-sinulariolide acetate, 11-acetoxy-15(17)-dihydroxinulariolide, 7,8-epoxy-11-sinulariolide acetate, and 3,4:8,11-bisepoxy-7-hydroxycembra- 15(17)-dihydro-1,12-olide	Cytotoxic	1
Sinulariolide, flexibilide, dihydroflexibilide, and organic extracts	Cytotoxic, Algicidal, Cardiac vasorelaxant, Feeding deterrents	2 3
Flexibiolide, dihydroflexibiolide sinularin, dihydrosinularin, sinuflexolide, sinuflexibilin, alcyonin, dihydrosinuflexolide, sinuflexin, and toxic extracts		4 5
Flexibilide, sinulariolide, and 11-epi-sinulariolide	Antimicrobial	6
Flexibilide	Algaecide	7
Flexibilide, 7,8-deoxyflexibilide and crude extracts	Anti-inflammatory	8
Toxic extracts	Ichthyotoxic	9
Flexibiolide, sinulariolone, 8,11-epoxy cembranolide, lobatrientriol, acetoxylobaoxide, lobatrienolide and flexibilene	Allelopathic	
Aqueous extract	Unknown	10
Flexibilide and dihydroflexibilide	Antifouling	11
2-Phenylethylamides	Allomones*	12
Phospholipase A ₂	Atrial stimulants	13
Mycosporine-like Amino Acids (MAAs)	Toxic compound (possibly cytolytic)	14
5,8-epidioxysterols, and a cinnamide compound	Photoprotective (sunscreens)	15
	Unknown	16

* Any chemical released by one species for defense that affects the physiology of another species.

1 Hsieh *et al.*, 2003

2 Aceret *et al.*, 1995; Aceret *et al.*, 1996; Aceret *et al.*, 2001; Alino *et al.*, 1992

3 Kumar and Lakshmi, 2006; Maida *et al.*, 1993; Wahl, 1989

4 Duh *et al.*, 1998a; Duh *et al.*, 1998b; Anjaneyulu and Sagar, 1996

5 Coll *et al.*, 1982a; Kusumi *et al.*, 1988; Weinheimer *et al.*, 1977

6 Aceret *et al.*, 1998; Maida *et al.*, 2001; Michalek-Wagner and Bowden, 1997; Mayer and Gustafson, 2004; Su *et al.*, 2005

7 Norton and Kazlauskas, 1980; Buckle *et al.*, 1980

8 Coll and Sammarco, 1983; La Barre *et al.*, 1986; Uchio *et al.*, 1988

9 Coll *et al.*, 1982a; Maida *et al.*, 1995a; Maida *et al.*, 1995b; Maida *et al.*, 2001; Sammarco *et al.*, 1983

10 Anjaneyulu *et al.*, 1998; Guerrero *et al.*, 1995; Hamada *et al.*, 1992; Herin and Tursch, 1976; Mori *et al.*, 1983

11 Maida *et al.*, 2006

12 Schulte *et al.*, 1991

13 Kazlauskas *et al.*, 1980

14 Nevalainen *et al.*, 2004

15 Michalek-Wagner, 2001

16 Anjaneyulu *et al.*, 1998; Yu *et al.*, 2006

Slattery, 2005). The earliest isolated terpenoid was sinulariolide (Tursch *et al.*, 1975). This compound and two other compounds from this species that were isolated later, sinularin and dehydroxysinularin, had potential anticancer activity (Yates and Carlson, 1992). The metabolite 7,8-deoxyflexibilide that is present in low concentrations in *S. flexibilis*, was found to be toxic for the Japanese medaka fish *Oryzias latipes* (Uchio *et al.*, 1988). Alcyonin was purified from an Okinawan *S. flexibilis* (Kusumi *et al.*, 1988) with cytotoxic activity against Vero cells (kidney cell cultures from monkey). The same species was later reported to yield three diterpenes, lobatrientriol, acetoxylobaoxide, and lobatrienolide (Hamada *et al.*, 1992), but no biological activities were reported.

Many of the compounds are known to play important ecological roles in the defense against predation (feeding deterrence and ichthyotoxicity) and competition for space via allelopathy (reviewed by Coll, 1992; Sammarco and Coll, 1988). Diterpenes from *S. flexibilis*, for instance, were found to inhibit the development of eggs and larvae of two stony corals *Acropora formosa* and *Porites cylindrica* *in vitro* (Aceret *et al.*, 1995). The release of toxic secondary metabolites of this species into the surrounding water (Coll *et al.*, 1982a; Coll *et al.*, 1982b) promoted inhibition of growth and mortality of neighboring scleractinian corals by altering their photosynthesis and respiration rates (Radhika *et al.*, 2002; Takaki *et al.*, 2003). Low concentrations (1-5 mg.L⁻¹) of some compounds (mainly flexibilide) from *S. flexibilis* have also been shown to cause expulsion of nematocysts and zooxanthellae, and subsequent death in scleractinian corals (Aceret *et al.*, 1995; Aceret *et al.*, 1998).

These molecules, although lipophilic, are highly soluble in seawater (e.g. Buckle *et al.*, 1980),

and as allelopathic or anti-fouling agents are selectively absorbed onto biomembranes of fouling organisms. It has been found that the water around soft corals, specifically *S. flexibilis*, can contain between 1 to 5 mg.L⁻¹ of flexibilide and dihydroflexibilide (Coll *et al.*, 1982a; Coll and Sammarco, 1983). This range is the concentration of toxin required to induce mortality in several scleractinian corals enabling the soft coral to exert influence on neighboring organisms in competition for space or fouling interactions (Maida *et al.*, 2006). The identification of the potent algaecide 11-epi-sinulariolide from *S. flexibilis* (Michalek-Wagner and Bowden, 1997) provides further evidence for the potential efficacy of released metabolites as anti-fouling agents. An antimicrobial compound described by (Averet *et al.*, 1998) is expected to be used as future antibiotic.

The effects of *S. flexibilis* on specific neighboring scleractinian corals are variable. That is, while some colonies of a given species of scleractinian suffer deleterious effects when interacting with *S. flexibilis*, other colonies in the same situation might not be affected. Although this variability of effects can be explained by an individual resistance of the scleractinian coral to allelochemicals, it may also be due to the allelopathic potential of a given *Sinularia* colony, i.e., the allelochemical content of the soft coral involved in the interaction (Maida *et al.*, 1993).

Because *Sinularia flexibilis* is highly toxic (Sammarco and Coll, 1987), it is rarely overgrown by epibionts (bacteria or algae: Aceret *et al.*, 1998; Wahl, 1989). Studies showed that antimicrobial properties of the diterpenes help protect the coral from competitors and predators. Two of the five tested diterpenes inhibited the growth of gram-positive bacteria, suggesting that this set of

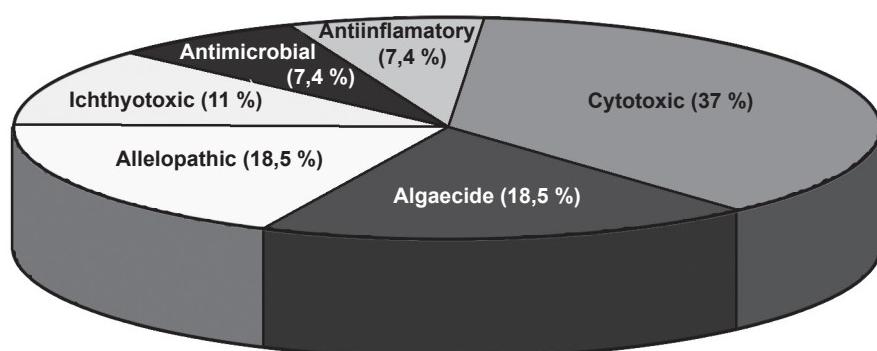


Figure 2.: Percentage of main activities of bioactive metabolites from *S. flexibilis* based on No. of reports.

compounds may be an important source of new antibiotics (Clark, 2000). Nonetheless, of the many diterpenes isolated from *S. flexibilis*, only one (Aceret et al., 1998) has been studied for antibiotic purposes. *Sinularia flexibilis* yields a dichloromethane extract that typically contains approximately 8 mg of flexibilide (sinularin), 6 mg of dihydroflexibilide and 2 mg of sinulariolide per gram of coral dry weight (200-800 mg.kg⁻¹ wet weight) (Maida et al., 1993). Flexibilide, the major terpene isolated from *Sinularia flexibilis* (e.g. Aceret et al., 2001) is a potent vector in allelopathy (Coll and Sammarco, 1983); it exhibited anti-inflammatory and anti-arthritic activity in rats (Norton and Kazlauskas, 1980), a property similar to the anti-inflammatory drug phenylbutazone (Kazlauskas et al., 1978). Flexibilide was also found to be an effective oral anti-inflammatory agent against rat paw oedema at 20-100 µmol.kg⁻¹ doses (Buckle et al., 1980). In the same study, an advantage of flexibilide compared to betamethasone valerate, an anti-inflammatory drug, was that the rats treated with flexibilide did not loose weight, showed no side effects, being as healthy as untreated rats.

A broad range of biological activities have been reported for sinulariolide as being an algaecide with antifouling properties (Tursch, 1976); it also showed marginal cytotoxic activities against a number of cell lines (proliferating cancerous cell cultures) (Sui-jian et al., 2002). Flexibilide, dihydroflexibilide, and sinulariolide were shown to be cardioactive, producing vasorelaxant responses in the isolated rat tissues, which may be useful for improved treatment of cardiovascular disease, especially heart failure (Aceret et al., 1996). Flexibilide and sinulariolide were found to be effective potential anticancer agents (Weinheimer et al., 1977); both compounds also exhibited antimicrobial activity and inhibited growth of Gram-positive bacteria (Aceret et al., 1998); hence, they were reported as antibacterials being at preclinical research in 1998 (Mayer and Lehmann, 2000). More studies on this species revealed that organic extracts of *S. flexibilis* inhibited coral larvae settlement (Maida et al., 1995a; Maida et al., 1995b). A further study identified 11-epi-sinulariolide as the active algaecide exhibiting highly bioactive characteristics at many levels (Michalek-Wagner and Bowden, 1997). In addition, cembranoid diterpenes isolated from *S. flexibilis*: sinuflexolide, dihydrosinuflexolide, sinuflexibilin, and sinuflexin showed significant cytotoxicity in human lung adenocarcinoma,

human colon adenocarcinoma, human epidermoid carcinoma, and mouse lymphocytic leukemia cell cultures (P-388: Duh et al., 1998a; Duh et al., 1998b). An atrial stimulant compound was also reported in *S. flexibilis* (Kazlauskas et al., 1980).

Sinulariolone, a new highly oxygenated cembranoid, was obtained from a Philippine collection of *S. flexibilis* (Guerrero et al., 1995), and the trihydroxy cembranolide lactones, flexibiolide and dihydroflexibiolide were isolated from an Indian collection of *S. flexibilis* (Anjaneyulu and Sagar, 1996). Besides, five cembranolides with three new analogues from this species were isolated (Hsieh et al., 2003), for which the cytotoxicity was also confirmed. Phospholipase A2, a toxic enzyme with a defensive role present in tissue homogenate of *S. flexibilis* was also identified (Nevalainen et al., 2004). Conclusively, the antimicrobial activity of *S. flexibilis* diterpenes will not only add information to the growing pharmaceutical knowledge on marine compounds, but also indicate their potential as a source of antibiotics (Aceret et al., 1998; Maida et al., 1993).

In addition to the terpenes, *S. flexibilis* is also rich in sterols. Six new sterols were isolated and characterized in this species (Jia et al., 2006; Uchio et al., 1988). The marine sterols were reported to show a variety of biological and pharmacological activities (Faulkner, 1997; Miyaoka et al., 1997); those compounds were suggested to be potential candidates for anti-allergic drugs development (Jin et al., 2005).

NATURAL SUNSCREENS

Mycosporine-like amino acids (MAAs) in corals are an important component of their photo-protective system against harmful UV radiation in shallow waters (e.g. Scripps, 2007). It has also been found that MAAs are biological antioxidants in coral tissue and zooxanthellae (Yakovleva et al., 2004). The unique physical and chemical properties of MAAs as natural sunscreens prompted an investigation of their use in health-care applications and in the formulation of cosmetic products (Volkman, 1999). MAAs in *S. flexibilis* are composed of six different components, with palythine as the major one (95 %; Michalek-Wagner, 2001). The major property of MAAs as photoabsorbents suggest potential commercial application in suncare products for skin protection and protection of non-biological materials as photostabilising

additives in the plastic, paint and varnish industries (Bandaranayake, 1998).

In this review, the number of publications related to particular toxic activities were counted. In figure 2 it is shown that most activities reported are cytotoxic. This suggests that these compounds are expected to be promising anticancer drugs.

THE DEMAND FOR CORAL BIOMASS

Despite the fact that many natural products from marine invertebrates are promising drugs or drug leads, the inadequate supply of coral biomass as a raw material has delayed the development of these agents (Fenical, 2006). This has been a major constraint in the development of the bioproducts from corals (e.g. sarcophytol: Fahmy *et al.*, 2004). A critical step is the inclusion of a sustainable, industrially feasible supply in order to overcome this main limitation and ensure a regular pathway of preclinical-clinical investigations, not to mention the market demand, for which a preliminary estimation is made below.

Possible means to overcome the supply problem are environmentally amenable ways of obtaining adequate supplies of the compounds of interest; these include aquaculture, cell culture, analogue development, chemical synthesis, and genetic manipulations (Clare *et al.*, 1999). Synthesis of the bio-products as the commercial source of choice for pharmaceutical industry allows control of all aspects of production. But, unlike terrestrial bio-compounds, many bioactive marine natural products, particularly those used in the pharmaceutical field, are extremely complex in structure, and require intensive multi-step processes that are not amenable to economic, industrial-scale synthesis. Therefore, the complexity of the marine-derived chemical structures, difficulty to develop, low yields (Müller *et al.*, 2000), and expensiveness can limit the development of synthesis processes. However, synthesis of two compounds from *S. flexibilis*, flexibilene (a short and simple path) and alcyonin (in 11 steps), were reported by McMurry *et al.* (1987) and Corminboeuf *et al.* (2003).

The establishment of cell lines from marine invertebrates has encountered obstacles; no single valid marine invertebrate cell line had been developed by 1998 (Rinkevich, 1999). For instance, in only one study (Frank *et al.*, 1994), despite the establishment of long-term

cell cultures from ten taxa of marine cnidarians (including octocorals), secondary cell cultures from corals were not fulfilling as in most cases, cells were maintained until 1 year without any signs of multiplication. However, five studies relating to the development or improvement of cell cultures from corals have been published from 1999-2004, which represented short-term experiments

(<1 month) with significant implications for holding cnidarian cells (including corals such as the genera *Stylophora*, *Porites*, *Dendronephthya*, and *Nephthya*) *in vitro* (reviewed by Rinkevich, 1999). For example, a maximum of 300 hours of survival of cultivated cells of hard coral species was reported (Kopecky and Ostrander, 1999), but did not succeed in developing continuous proliferating cell cultures. Although some encouraging reports on cnidarian cell culture systems have been presented, the lack of vital information regarding cell requirements and their physiology and biochemical patterns *in vitro*, as well as improper comparisons between vertebrate-invertebrate cell requirements might account for the failure (Rinkevich, 2005).

A long-term laboratory culture of the coral juvenile (planula or coral larvae) from the symbiotic hard coral *Acropora tenuis* has been developed recently (Watanabe *et al.*, 2007). The authors state that since the adult corals were difficult to rear in laboratories, they developed conditions for laboratory culture of symbiotic juveniles of *A. tenuis* by modifying culture conditions, with the purpose of using the coral juveniles in molecular and ecotoxicological studies. The coral juveniles maintained for at least three months, and exhibited size increment during that period. This could be encouraging for culture of hard-farming coral species.

Since most of these bioactive metabolites are found at low concentrations, large collections of the source coral are needed to isolate sufficient compounds for clinical trials (Kerr *et al.*, 2004; Proksch *et al.*, 2003). For drug development or production, it is often not economical and would be environmentally destructive and expensive to supply drugs by large-scale harvesting from the environment (Davies, 1995). Aquaculture, as an alternative to natural harvest requires major advances in culture techniques. In some cases, it might be the only way to obtain sufficient amounts of compounds (Munro *et al.*, 1999). A series of studies have been done on aquaculture

(mari- and/or lab-culture) (e.g. Wahl (1989) in aquarium systems; Bongiorni *et al.* (2003) *in-situ*) to reduce the need for coral collection for ecotoxicological studies (Davies, 1995). Successful aquarium rearing of corals has been reported from hobby and public aquaria and we think that aquaculture could be a satisfactory solution for the supply of certain species and phyla of marine invertebrates. This is already in large scale for many corals to provide corals for the ornamental trade. It has been shown to be a feasible and manageable technology to meet, in part, the needs for commercial drug supplies (Mendola, 2003; Proksch *et al.*, 2003). Nevertheless, potential problems related to mass culture such as loss of culture facilities and stocks by storms or by

diseases are possible scenarios that might make reliable continuous supply more or less difficult (Proksch *et al.*, 2003). A further advanced biotechnological approach, the genetic manipulation, by cloning and expression of the respective biosynthetic genes (if they are known, which they are mostly not) provides the opportunity to spread biosynthesis of secondary metabolites. A summary of the advantages and disadvantages of various approaches of mass production of the coral is presented in table 2. Considering the pros and cons for each approach, it is concluded that those approaches with proven feasibility (captive aquaculture and mariculture) are more promising in short term. These approaches also provide valuable

Table 2: Advantages and disadvantages of various means of mass production of the coral.

Approach	Advantage	Disadvantage
Aquaculture (closed system)	Environmental parameters are controllable; growth kinetics and the effects of culture conditions on the biosynthesis of the compounds is easily studied (Khalesi <i>et al.</i> , 2007); year-long cultivation (Sipkema <i>et al.</i> , 2005); feasibility is proven (e.g. Bruckner, 2002; Sipkema <i>et al.</i> , 2005).	Possibly expensive, time consuming, and space intensive; requires knowledge of species-specific cultivation parameters.
Mariculture (open system)	Cost-effective (Mayer and Gustafson, 2004); bulk supply of the compounds; feasibility is proven (Bongiorni <i>et al.</i> , 2003).	Complete control of environmental parameters and diseases not possible; possible destruction of culture facilities by storm; seasonality (Sipkema <i>et al.</i> , 2005).
Tissue or cell culture	Establishing cell lines as models for <i>in vitro</i> production of bioactive metabolites; to study the factors controlling production to enhance process optimization.	Not successful to establish long-lasting, proliferating cell lines up until now;
Organic synthesis	Allows control of all aspects of production; successfully done for a number of compounds.	Multi-step process; not applicable for all compounds; low yield; expensive (Proksch <i>et al.</i> , 2003).
Genetic manipulation	Possibility to identify, isolate, clone, and express the genes for production of the compounds in a heterologous host.	The biosynthesis of the target compound is mostly not or poorly known; expensive; low feasibility; lack of effective techniques.

knowledge on coral biology to be used for biotechnological applications.

ESTIMATING THE ANNUAL DEMAND FOR *S. FLEXIBILIS*

The average content of the major antibacterial terpene (flexibilide) in *S. flexibilis* was found to be 5×10^{-3} kg.kg⁻¹ coral dry weight (Aceret *et al.*, 1998; Coll *et al.*, 1982a; Coll and Sammarco, 1983.). The yearly demand for other commercial drugs varies from 1–5 kg for sponge halichondrins up to 45,000 tonnes for penicillin (Hunt and Vincent, 2006; Bruggink, 2001). It can, therefore, be estimated that the annual coral need for flexibilide production should lie between 10^3 to 10^4 kg dry weight (10^4 – 10^5 kg wet weight), depending on the type of the required compound.

Assuming a regular annual supply of 10^{11} kg of the coral biomass yielding 50 tonnes flexibilide.ha⁻¹.y⁻¹ from a cultivation system, we would need an area of 0.2×10^8 hectare. These very preliminary estimates of the annual biomass production for preclinical-clinical trials suggest that an annual production equates to huge amount of the coral that apparently can be partially supplied by aquaculture, as is also true for sponges (Munro *et al.*, 1999). It has been estimated that sponge halichondrins necessitate an annual harvest of 3,000–17,000 tonnes of

the source sponges (Hunt and Vincent, 2006). Even though, large-scale production of sponge metabolites by generation of biomass is not probable in many cases (Sipkema *et al.*, 2005). Therefore, because of apparent uncertainty for large-scale coral cultivation, it can be directed to be an alternative for production of specialty drugs (e.g. anticancers) which have a lower market demand than antibiotics (halichondrins vs. penicillin).

A more precise estimate together with actual production variables and economics can be made via the required quantity of the compounds. *In vitro* culture of the source coral under controlled conditions is considered to be a viable method for supplying metabolites for drug development (e.g. Davies, 1995), and also a particularly challenging opportunity for marine bioprospecting to discover new bioproducts (Pomponi, 2004); such a system also allows growth parameters such as light levels and water flow, as well as compound biosynthesis to be optimized (Sipkema *et al.*, 2005). Therefore, for *in vitro* culture to succeed, it is vital to develop appropriate biological regimes that promote growth and biosynthesis of the target metabolites (e.g. Duckworth, 2004). In addition, in order to develop models for aquaculture, factors such as the standing stock of the organism, its growth and the factors that affect growth should be clarified (Pomponi, 1999).

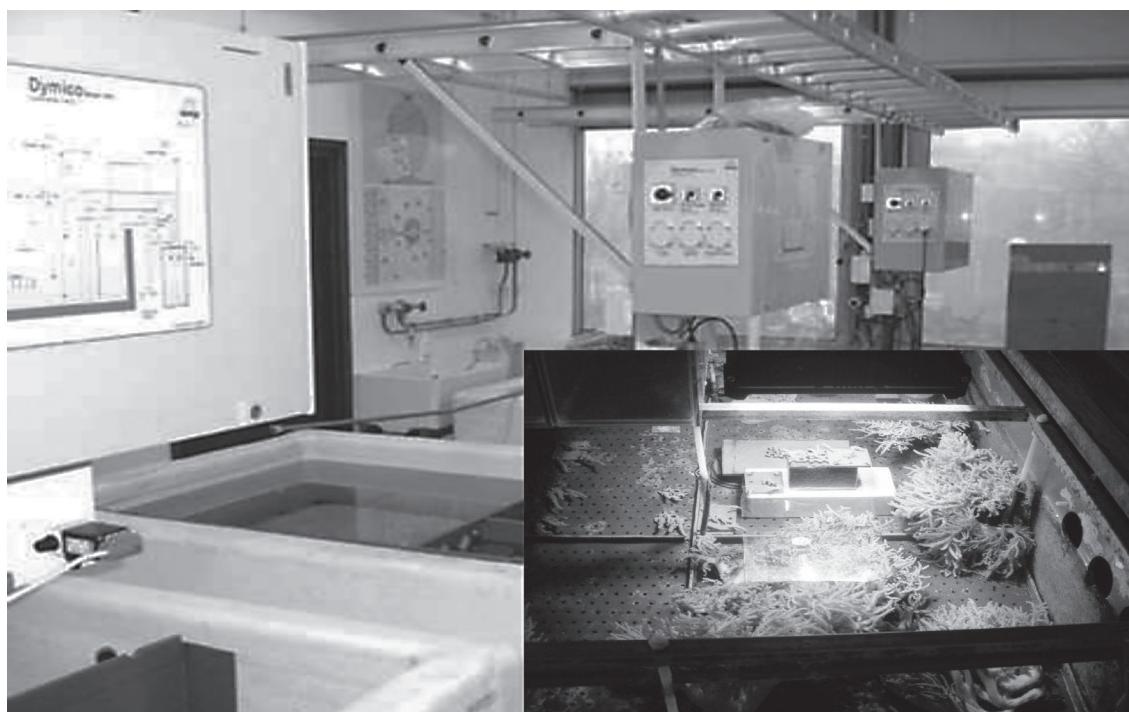


Figure 3: Eco-Deco cultivation system at Wageningen University, Marine Biotechnology lab.

CULTIVATION OF *S. FLEXIBILIS*

With the goal of creating a sustainable resource, through a series of studies on the cultivation of *S. flexibilis* in captivity, it was shown that colonies of this soft coral are able to fully adapt to laboratory conditions in a relatively short period. The data on growth kinetics (Khalesi *et al.*, 2007a) and metabolite biosynthesis demonstrate that *S. flexibilis* responds convincingly to the controlled conditions, being able to produce flexibilide, which, for instance, is influenced by light intensity (Khalesi *et al.*, 2007b). Optimum specific growth rate (μ) of *S. flexibilis* was found at a range from 100 to 400 $\mu\text{mol quanta.m}^{-2}.\text{s}^{-1}$. More or less constant μ over a range of light intensities was attributed to photoacclimation of the coral. Our long-term data also showed that in the absence of light, even with addition of food, the coral was not able to grow nor to survive. Similarly, corals kept under optimal light without a continuous supply of ambient nutrient supply, were incapable to grow and survive. On the other hand, both colonies of *S. flexibilis* incubated at normal irradiances with or without extra feeding with *Artemia* nauplii, grew well using available nutritional sources.

The culture system (Eco-decosystems, Dymico-Model 1000, $\pm 1,300 \text{ L}$, Figure 3) was also a mechanised system that could be developed to a large-scale stocking-type culture system.

Our findings show that *S. flexibilis* could be grown successfully in small-scale trials and that the flexibilide continued to be biosynthesised, even after several years of culture. In addition, an advantage of *S. flexibilis* with a branching growth form is that more small fragments can

be collected from the parent colony, without affecting its ability to recover from the collection. Another benefit of this species compared to some other symbiotic corals is its absolute light-dependency (Khalesi *et al.*, 2007c) independent of manual feeding in captivity.

Similarly, sustainable harvest has allowed researchers to obtain sufficient supplies of a gorgonian coral (*Pseudoterigorgia elisabethae*) over a 15-year period without devastating local populations, ultimately leading to purification of a highly profitable product, pseudopterosins, by ensuring an adequate supply (Bruckner, 2002). It was also shown that a cultured soft coral *Eleutherobia caribaeorum* produces the secondary metabolite (eleutherobin) in captivity (Taglialatela-Scafati *et al.*, 2002). Considering the fact that no pharmaceutical company has yet relied solely on aquaculture for bulk supply of a compound (Hunt and Vincent, 2006), the above-mentioned evidences indicate that coral culture opens the way to aquaculture production of interesting pharmacological agents.

ECONOMICAL CONSIDERATIONS

The economics of soft coral farming is affected by many variables such as location, its size and market; the costs will vary greatly with the region (Ellis and Samson, 1998). To obtain insight in the economics of *S. flexibilis* mass production, our estimate is based on costs of sponge *ex-situ* cultivation (Sipkema *et al.*, 2005). This is because both the cultivation system and regional characteristics were similar. The feeding costs are excluded (because of the

*Table 3: Summary of expenses for two approaches of coral mass production, extracted from Sipkema *et al.* (2005) with some changes (left column). The right column displays the costs of coral mariculture (Ellis and Samson, 1998; www1).*

Ex-situ aquaculture		Mariculture	
Equipment	Price (€)	Equipment	Price (€)
Cultivation tank (5 m ³)	10,000	Green house (25×12)	9,500
Laboratory	2,000 m ⁻²	Heat pump	3,390
Production space	800	Sump	300
Storage house	500	Pumps	600
Lighting bulb/tank	100	Electrical	850
Electricity/12 h. d ⁻¹ /bulb	220 y ⁻¹	Boat and motor	1,400
Office	1,200 m ⁻²	Land lease (if applicable)	2,400
Employee	37,000	Other costs (incl. employee)	12,000
Total	€ 51,820 y⁻¹	Total	€ 30,600 y⁻¹

phototrophy of the coral) and instead, lighting expenses are included. The raw costs of our closed aquaculture (*ex-situ* cultivation) have been compared to available respective recourses on coral commercial mariculture (Ellis and Samson, 1998; www1).

For the production of 1 kg of flexibilide, a minimum coral biomass of 2×10^3 kg (wet weight) is needed. If a cultivation tank (5 m³, table 3) yields 20 kg coral wet weight annually, the cost per kg flexibilide approximates € 5×10^6 y⁻¹. Although still very high, this is lower than that of € 1.9×10^7 y⁻¹ for production of sponge halichondrins through *ex-situ* cultivation (Sipkema *et al.*, 2005). It should be noted that the coral culture for drug development, because of expensive expenses, might be directed towards the production of high-value pharmaceuticals.

Considering the lower annual cost for coral mariculture (table 3), the cost for the coral mariculture per kg of flexibilide per year will be lower. Based on very rough calculations of the expenses (table 3) and also estimations on flexibilide production, an economically feasible technique to culture coral on a relatively larger scale, can be a combination of off-sea (land-based) and controlled cultivation, depending on regional facilities. In that case, while taking advantage of lower costs, environmental parameters are also controlled.

CONCLUSIONS

In this review, the studies on the application of secondary metabolites from the soft coral genus *Sinularia* have been broadly surveyed. The overview, covering a period of over 30 years, shows that the soft coral *Sinularia flexibilis* has attained a high number of researches on its bioproducts. The evidence of novel and pharmacologically active compounds from *S. flexibilis* points out its potential biomedical significance for future therapeutic agents. As a consequence, an increased demand for such a pharmacological and toxicological model for screening as well as for in depth studies might lead to overexploitation of the corals' natural reservoir. These facts, together with the evidence provided in this review, would present a demand for sustainable approaches of long-term exploitation. This review concentrates on the pharmacological potential of some highly potent compounds, as a group called toxins, isolated from *S. flexibilis*, as well as the coral's

potential for *in vitro* cultivation. Conclusively, growing the coral under controlled conditions with further expansion of the knowledge base for their aquaculture is one alternative by which the biomass required is supplied.

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